In Ovo Exposure to *o,p′*-DDE Affects Sexual Development But Not Sexual Differentiation in Japanese Medaka (*Oryzias latipes*)

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Despite being banned in many countries, dichlorodiphenyltrichloroethane (DDT) and its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) continue to be found in fish tissues at concentrations of concern. Like *o,p′*-DDT, *o,p′*-DDE is estrogenic and is believed to exert its effects through binding to the estrogen receptor. The limited toxicologic data for *o,p′*-DDE suggest that it decreases fecundity and fertility of fishes. We conducted an egg injection study using the d-rR strain of medaka and environmentally relevant concentrations of *o,p′*-DDE to examine its effects on sexual differentiation and development. The gonads of exposed fish showed no evidence of sex reversal or intersex. However, other gonad abnormalities occurred in exposed individuals. Females exhibited few vitellogenic oocytes and increased atresia. Male testes appeared morphologically normal but were very small. Gonadosomatic index values for both sexes were lower for exposed fish. Our observations of abnormal female and very small male gonads after *in ovo o,p′*-DDE exposure may be indicative of effects on early endocrine processes important for normal ovarian and testicular development. *Key words:* egg injection, medaka, *o,p′*-DDE, sexual development, sexual differentiation, xenoestrogen.
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In ovo exposure of fish to the synthetic estrogen ethinyl estradiol can cause genetic males to sexually differentiate as phenotypic females (Papoulias et al. 1999). Presumably, the binding of estrogen to the estrogen receptor (ER) initiates the little-understood process of establishing the female phenotype in teleost fishes. Because many man-made chemicals bind to the ER, the question arises of whether environmentally exposed male fish are at risk of being feminized.

Although use of dichlorodiphenyltrichloroethane (DDT) is banned in much of the world, the parent compound and its metabolites persist in the global environment, throughout the food chain, and thus remain a concern (Brown 1997; Munn and Gruber 1997; Schmitt et al. 1999; Simonich and Hites 1995). DDT and its metabolites are highly lipophilic and therefore easily bioaccumulate in fish. Ungerer and Thomas (1996) demonstrated that breeding females will accumulate *o,p′*-DDT within the triglyceride-rich oil globule of the oocyte. Here, the *o,p′*-DDT remains sequestered until egg fertilization, after which it becomes available to the developing embryo. The oil globule is generally the last endogenous nutritional source used by the sac fry before they switch to exogenous feeding (Heming and Buddington 1988). Thus, for many species, the greatest exposure to the developing embryo from hydrophobic estrogenic chemicals occurs during sexual differentiation (i.e., shortly after hatch).

Despite the diminished environmental presence of DDT over the last 30 years, DDE (dichlorodiphenyldichloroethylene) metabolites are still commonly detected (Adeshina and Todd 1991; Brown 1997; Hellou et al. 1995; Schecter et al. 1997). DDE is a breakdown product of DDT, a recognized endocrine system modulator. However, very few laboratory studies of the effects of DDE have been published, and of these, most report on the *p,p′*-DDE isomer. The *p,p′*-isomers are far more common than the *o,p′*-isomers, because technical-grade DDT consists of approximately 80% *p,p′*-DDT and 20% *o,p′*-DDT. The *p,p′*-isomers are considered androgen receptor antagonists and not xenoestrogens (Kelce et al. 1995).

Although *o,p′*-DDT has long been recognized as estrogenic (Bitman et al. 1968; Kupfer and Bulger 1976), relatively little is known about its metabolite *o,p′*-DDE. However, like *o,p′*-DDT, *o,p′*-DDE is believed to work through the ER and to have a similar ER agonist potency (Donohoe and Curtis 1996), and exposure to *o,p′*-DDE has been associated with decreased fecundity and fertility and increased early oocyte atresia (Hose et al. 1989). Recently, Wells and Van Der Kraak (2000) showed that *o,p′*-DDE and *o,p′*-DDT will also bind to the androgen receptor in goldfish (*Carassius auratus*) but not rainbow trout (*Oncorhynchus mykiss*) testes at approximately half the affinity of *p,p′*-DDE. *o,p′*-DDE has even been shown to bind to the progesterone receptor in the eggshell gland mucosa of egg-laying ducks and domestic fowl and to the endometrium of the rabbit uterus (Lundholm 1988). Furthermore, DDT can be metabolized to DDE by the developing fish embryo (Atchison and Johnson 1975).

The objective of this study was to investigate the effects of *o,p′*-DDE on sexual differentiation and development. We accomplished this through injection of environmentally relevant concentrations of *o,p′*-DDE into early-stage embryos of medaka (*Oryzias latipes*).

Materials and Methods

Test organism. The d-rR strain of medaka was a generous gift of Akihiro Shima (University of Tokyo, Tokyo, Japan). Broodstock were maintained at the Columbia Environmental Research Center (CERC) in well water under an 18:6 hr light:dark photoperiod at 27°C. In this strain, either R (orange-red) or r (white) is carried by the X or Y chromosome, respectively. White females (X^rX^r) crossed with orange-red males (X^rY^R) produce approximately equal numbers of white daughters and orange-red sons. Crossovers or genetic imbalances occur at a rate of about 0.005–0.5%, making coloration a reliable marker of genetic sex (Hishida 1965).

In addition to the genetic marker, there is distinct sexual dimorphism in medaka. Males bear a notched dorsal fin, an anal fin with a convex and serrated posterior margin, and an elongate penultimate anal fin ray that give their anal fins a square appearance. In addition, papillar processes form on the rays of the anal fin in breeding males. Females lack the dorsal notch, and their anal fins are more concave, smooth at the margin, and lack extended rays.

Injection exposure. **Preparation.** Details for embryo exposure and rearing are provided in Papoulias et al. (1999). Embryos were collected

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